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Gene Therapy

Principal Investigator: Bakay, Roy A
Grant Number: 1R01NS046612-01A1
Title: Stem Cells in CNS Transplantation

Abstract: Stem cells offer tremendous promise for the future of transplantation. We propose examining embryonic stem cells (ESC) in monkey allografts. We will compare dopaminergic enriched ESC to fetal mesencephalic (FM) neurons in their ability to survive, innervate, and restore lost function in the best animal model of PD, the MPTP treated monkey. The primate is essential for this study to test the hypothesis that replacement strategy must completely reinnervate the very large volume of the monkey striatum. Recently clinical trials have indicated that dopaminergic (DAergic) replacement with FM neurons can cause severe debilitating dyskinesia. It is then imperative to have a clear understanding of how a DAergic enriched ESC replacement strategy affects l-dopa-induced dyskinesia (LID). In this regard, we will also compare the effects of FM transplants and DAergic enriched ESC upon the dyskinesia profile of MPTP monkeys. The potential to induce or diminish dyskinesia will be tested with the best model of dyskinesia (primate LID model). The key problem of parkinsonian transplantation with fetal or stem cells grafts is the incomplete reinnervation of host striatum. Like the FM transplant patients, focal areas of relative hyperdopaminergic activity should render these monkeys highly susceptible to LIDs. Thus to optimize reinnervation and functional recovery while minimizing the potential for dyskinesia, we will also treat DAergic enriched ESC with glial cell line-derived neurotrophic factor (GDNF) delivered via a lentiviral vector. The lenti-viral vector is critical to this hypothesis because of the proven ability to transfect the entire striatum and act not as a point source but as a volume source to stimulate reinnervation. Intraparenchymal GDNF released diffusely throughout the entire striatum should act as a developmental cue for these immature cells to extend DAergic processes throughout the striatum as well as provide neuronal rescue for dopaminergic neurons in the pars compacta of the substantia nigra. Sufficient subjects and multiple controls are included to insure proper interpretation of the data. The present series of experiments serves to provide the essential preclinical data needed to help determine the utility of nonhuman dopaminergic enriched stem cells. -

Principal Investigator: Bohn, Martha C
Grant Number: 5R01NS031957-08
Title: GENE THERAPHY FOR PARKINSON'S DISEASE

Abstract: The long-term goal of this project is to develop novel gene therapies for neurodegenerative diseases. In the previous support period, we focused on adenoviral (Ad) vectors to deliver the gene encoding GDNF (glial cell line-derived neurotrophic factor). Ad-GDNF injected into either the substantia nigra or striatum of a progressive degeneration model of Parkinson's disease protected dopaminergic (DA) neurons against cell death induced by the neurotoxin 6-OHDA. Ad-GDNF injected into the striatum also prevented the acquisition of behaviors and molecular changes that occurred in DA deficient young and aged rats. This proposal focuses on the hypothesis that anti-apoptotic gene delivery will also protect DA neurons in vitro and in vivo and have a synergistic effect with delivery of neurotrophic factor genes. Viral vectors harboring genes that block specific apoptotic death pathways, including XIAP, a dominant-negative caspase-9, bcl-2 and bclxl will be studied for effects on survival and function of DA neurons either alone or in combination with neurotrophic factors, GDNF or neurturin. Genes will be delivered to DA neurons in culture and in rat brain using helper free HSV:AAV hybrid amplicon vectors. These vectors will incorporate bidirectional expression cassettes that drive both the therapeutic gene and the cellular marker gene, green fluorescent protein, to permit specific evaluation of transduced cells. Expression will be controlled using the tetracycline responsive element such that transgene expression is "on" in the presence of tetracycline activator (TA) and in the absence of doxcycline (Dox). Vectors will be made in which TA is driven by a viral promoter of the DA cellular promoter, tyrosine hydroxylase (TH). Effects of the 'therapeutic' genes will be studied using non-neuronal cells, the DA cell line, MN9D, and primary fetal DA neurons treated with the neurotoxins, MPP+ or 6-OHDA or other cellular insults. In vivo effects of therapeutic genes will be studied in: 1) rats that have received grafts of fetal DA neurons, and 2) rats that have received a progressive 6-OHDA lesion of the nigrostriatal projection. Reversibility of effects will be studied by administration of Dox. Effects on DA neurons will be evaluated using quantitative morphometric and molecular techniques and behavioral evaluations. This project also aims to continue its evaluation of new generation viral vectors, including E2b deleted Ad, totally gutted Ad, and HSV:AAV amplicon, for stability and levels of expression in the nigrostriatal system. The studies involve collaborations among investigators at Children's Memorial Hospital and Northwestern Univ. Med. School and are relevant to the development of novel therapies for neurodegenerative diseases and injuries to the CNS. -

Principal Investigator: CASTRO, MARIA G
Grant Number: 5R01NS044556-03
Title: Gene therapy for chronic neurodegenerative disorders

Abstract: Parkinson's disease (PD) is a chronic neurodegenerative disorder. Although we do not yet understand its cause, there is extensive degeneration of nigro-striatal DA neurons. Powerful neurotrophic factors which could be used for the treatment of PD, like GDNF, have been described recently. The ultimate goal of this proposal is to develop novel high-capacity adenoviral systems for cell-type specific, inducible, long term, stable, and non-immunogenic delivery of neuroprotective genes to the brain for both experimental transgene expression in adult animals, and for the future treatment of chronic neurodegenerative diseases such as PD and Alzheimer's disease by gene therapy. Currently the use of adenovirus vectors has been limited by the low efficiency of transcriptional promoter elements currently used, which directly leads to the need to use higher doses of vectors, and the cytotoxicity and immunogenicity of viral proteins expressed from the genornes of first generation adenoviral vectors. We now wish to develop novel cell-type specific and inducible vectors, that will allow efficient, safe, and long-term gene delivery vectors for neurological gene therapy. We will construct high-capacity helper-dependent adenoviral vectors that express no adenoviral genes. We will utilize the powerful, astrocyte specific major immediate early murine Cytomegalovirus promoter, driving novel tetracycline-dependent transcriptional activators to achieve cell-type specific and regulatable expression of GDNF. The efficacy, cell-type specificity, and inducibility of these vectors will be tested stringently to assess their capacity to deliver cell-type specific and regulatable GDNF, and also to determine any potential side effects caused either by the vectors or the long term expression of powerful neurotrophic agents. The reagents and principles established by this work will be of substantial value to those with interests in the basic and clinical neurosciences, and will lead to the development of novel, efficient, and safe approaches for the treatment of human chronic neurodegenerative diseases. This research will facilitate the development of the tools needed to achieve long-lived, safe, cell-type specific, regulatable, non-cytotoxic transgene expression, and, ultimately, for the treatment of patients suffering from chronic neurodegenerative diseases.-

Principal Investigator: DURING, MATTHEW J
Grant Number: 1R01NS044576-01
Title: Somatic Cell Gene Transfer/Neurological & Clin Applics

Abstract: Gene transfer in the mammalian nervous system has been the primary research focus of our laboratory for the past decade. We are excited that this RFA has come at a time when the field is flourishing, yet clinical translation remains daunting, and much work needs to be done for ultimate success in the clinic. In this grant application we propose to focus on some of the more pressing needs using rat models of Parkinson Disease. Our first aim is to further develop more efficient and readily packaged and purified AAV vectors for clinical translation. Here, we will characterize and compare pseudotyped and chimeric AAV vectors and in addition develop novel reagents, including helper plasmids and protocols which can be used by the entire gene therapy community to more efficiently generate these vectors. Our preliminary data suggests that these new chimeric and pseudotyped vectors represent a significant advance above our current generation rAAV-2 vectors. Secondly, we will develop optimal expression cassettes with a focus on promoter; post regulatory sequences as well as elements like the human beta-interferon scaffold attachment region (SAR) to boost expression. Thirdly, we will further develop a regulatable system. We present in our preliminary data our latest generation bi-directional tet cassette with tandem minimal insulator sequences flanking the vector genome. Here we propose to use this vector as the starting point to develop a novel cassette with the use of KRAB-AB domain from kid-1 as a suppressor. Our fourth aim is the use of rAAV to over express PAEL receptor in the adult rat substantial nigra with characterization of the phenotype as a potential genetic model of Parkinson Disease. Finally, we propose the use of a picospritzer and in vivo single unit recording to develop methods for focal and electrophysiological mapped neuronal gene delivery. We will target the substantia nigra pars compacta, using AAV expressing wildtype parkin, as a potential therapy for parkin mutation associated, autosomal recessive Parkinson Disease (AR-PD) as modeled by the PAEL receptor over expressing rats as developed in specific aim 4.-

Principal Investigator: FEDEROFF, HOWARD J.
Grant Number: 5R01NS036420-08
Title: Improved HSV Vectors: Gene Transfer into Nervous System

Abstract: Gene transfer methods have created the opportunity for developing gene therapy for human neurological diseases such as Parkinson's Disease (PD). Since PD represents a group of clinically similar syndromes each triggered by a different mechanism we hypothesize the existence of a shared downstream pathophysiologic pathway. Our goal is to develop therapy for PD directed at a shared common node in the pathway. The elaboration of such neuroprotective gene therapy is contingent on the development of safe and efficacious gene transfer vectors that can express a therapeutic gene for a prolonged period in specific neuronal populations. Of the currently available vehicles for direct gene therapy only plasmid based herpes simplex virus (HSV) "amplicon" vectors have been demonstrated to both accommodate a large (9 kb) tyrosine hydroxylase (TH) promoter fragment and to provide highly selective gene expression in dopamine (DA) neurons in the substantia nigra. However, HSV amplicon vectors exhibit transgene silencing that is an impediment to one-time dosing for a chronic disease such as PD. Our data indicate that transgene silencing results from heterochromatin formation. One of the goals of this project is to subvert transgene silencing by altering the propensity of vector to form heterochromatin. In Specific Aim 1 we examine multiple different approaches to stimulate euchromatin formation, that chromatin state posited to support long term gene expression. A second issue pertinent to the development of PD gene therapy is to direct different therapeutic genes to each compartment of the diseased nigrostriatal pathway: dopamine neurons and target striatum. In Specific Aim 2 we will develop separate vectors which will afford direct expression of different gene products to each anatomical compartment. A third issue for successful PD gene therapy is evaluation in appropriate animal models of the disease. Specific Aim 3 will employ two animal models: Our novel a-synuclein mice which develop progressive nigrostriatal dysfunction, reduction of substantia nigra TH and hypokinetic activity; and our modified chronic MPTP model which produces striatal denervation, dopaminergic cell loss and a neurobehavorial syndrome. The proposed studies will yield optimized HSV vectors, provide a detailed understanding of their characteristics, and evaluate their effectiveness in mechanistically different models of PD. -

Principal Investigator: FEDEROFF, HOWARD J.
Grant Number: 5U54NS045309-03
Title: Parkinson's Disease Gene Therapy Study Group

Abstract: Parkinson's disease (PD) affects about 1 million people in North America. Medications, such as levodopa, and some surgical approaches are available for PD, but offer only symptomatic therapy. New information contribute to current optimism that gene therapy might correct the molecular disturbances of PD, alleviate the symptoms of the illness and/or in retarding disease progression. Setbacks in gene therapy for other diseases underscore the importance of a purposely deliberate and careful approach that demands substantial assurances of safety and potential efficacy in advance of human testing. It is this philosophy of conservatism that will characterize the activities of our group. A coordinated stepwise progression from basic research through exhaustive preclinical evaluation prior to clinical testing is required. A multicenter, multidisciplinary collaborative group (The PD Gene Therapy Study Group [PDGTSG]) has formed and seeks support for those activities that will lead to a large-scale clinical trial of gene therapy for patients with PD. The PDGTSG consists of three different components: Cores, Principal Projects, and Pipeline Projects. Core A. Administrative Core (PI: Dr. Federoff): Houses a Steering Committee, and Vector (Chair: Dr. Lowenstein), Human Subjects/Clinical Assessment (Chair: Dr. Kurlan), Bioethics (Chair: Ms. Greenlaw), Intellectual Property (Chair: Ms. Hunter) and Biostatistics Modules (Chair: Dr. Oakes). Provides for the coordination of budgeting, committee scheduling, reports, progress preparation, and interface with NINDS staff, the clinical, scientific and lay community. Core B. Biological Measurement Core (PI: Dr. Federoff: Functions in the application shared quantitative measurements. Houses the database and the bank of vector constructs used in all studies. Project I. "Enzymatic Gene Transfer in MPTP Monkeys" (PIs: Bankiewicz and Kordower) Will comprehensively evaluate two vector platforms (rHIV and rAAV), each transducing the identical AADC gene cassette in the standardized non-human primate model. Project II. "Trophic Gene Transfer in MPTP Monkeys" (PIs: Bankiewicz and Kordower) Will comprehensively evaluate two vector platforms (rHIV and rAAV), each transducing the identical regulated GDNF gene cassette in the standardized non-human primate model. PIPELINE PROJECTS 0PPs) Focus 1: 1reproved regulation of gene expression PP I. "Tet-Regulated Vectors for Parkinson's Disease" (PI: Bohn). PP II. "Engineering RNA Switches that Respond to Dopamine and its Analogs" (PI: Breaker). Focus 2: Development of new vector platforms for application in PD disease models. PP III. "High Capacity Gutless Adenovirus" (PI: Lowenstein). PP IV. "Development of Integrating HSV

Principal Investigator: GELLER, ALFRED I
Grant Number: 5R01NS043107-04
Title: Enhanced HSV-1 Vector Particles for Neural Gene Therapy

Abstract: This laboratory has developed a helper virus-free Herpes Simplex Virus (HSV-1) plasmid vector system for gene transfer into neurons. Using this system, we have begun to explore gene therapy approaches to specific neurological disorders, such as Parkinson's Disease (PD). We have shown that delivery of a HSV-1 vector that expresses human tyrosine hydroxylase into the partially denervated striatum in the 6-hydroxydopamine rat model of PD results in significant (64 percent) and long-term (1 year) behavioral recovery. Modifications to the vector particle have enhanced the utility of specific vector systems. First, the titers and infectivity of classical retrovirus vectors, lentivirus vectors, and other vector systems have been enhanced by pseudotyping with vesicular stomatitis virus (VSV) G protein. Recently, both we and other investigators have shown that HSV-1 vectors can be pseudotyped with VSV G protein and such vector particles can support gene transfer into neurons in the rat brain. Second, gene transfer has been targeted to specific types of cells by modifying the vector particle of classical retrovirus vectors or adenovirus vectors. Third, we have enhanced neural gene transfer and long- term expression by packaging vectors in the presence of mutations in specific HSV-1 proteins that affect the virion. The long-term goal of this proposal is to modify the HSV-1 vector particle to enhance its utility for human gene therapy of neurological disorders such as PD. The first specific aim will develop procedures for producing high titer HSV-1 vectors pseudotyped with VSV G protein. The second specific aim will target gene transfer to nigrostriatal neurons by modifying the HSV-1 vector particle to bind to specific receptors on these neurons. The third specific aim will enhance gene transfer and long-term expression by packaging vectors in the presence of mutations in specific HSV-1 proteins that affect the virion. These modified vector particles will be systematically characterized and then evaluated for gene transfer and expression in the rat brain. -

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 2R01NS032519-11A1
Title: Studies of Purified Dopamine Neurons

Abstract: Historically, there has been no good way to isolate DA neurons from other cells of the midbrain. Thus, missing DA neurons have been replaced by mixed cell populations following transplantation of embryonic midbrain tissue in animal models of disease and in Parkinson's patients. Although, in many cases, these transplants have provided long-term benefit, the presence of unwanted cells, such as glia, non-DAergic neurons, or even excessive numbers of DA neurons, has produced serious side effects, and in rare cases, even death. Discovering ways in which to segregate DA neurons from other cell types poses a significant challenge, but a necessary next step. In the present proposal, our plan is to take advantage of several new advances in the laboratory; including the recent cloning of 11kb human tyrosine hydroxylase gene promoter (hTH). This sequence accurately targets the expression of the reporter, green fluorescent protein (GFP) to DA neurons of the mammalian CNS. Because GFP can be directly visualized in live fetal DA neurons, this approach allows enrichment via flourescent activated cell sorting (FACS) for study in vivo and in vitro. Moreover, it is possible to adapt these purification methods to mouse stem and human progenitor cells using a lentiviral vector to transduce cells with the hTH-GFP transgene. Following their DA differentiation and FACS sorting, our goal is to study purified populations of engineered stem/progenitor-derived DA neurons in culture or after transplantation into the Parkinsonian rat. These models offer us a unique opportunity to determine the ideal number of DA neurons needed as well as the optimal conditions which contribute to their survival and growth following transplantation. Graft function will be assessed in live animals via behavioral testing and in vivo microdialysis which will be correlated with biochemical and anatomical (at the light and electron microscopic levels) changes following sacrifice. This work will hopefully lay the foundation for the development of therapeutic treatments for Parkinson's and other diseases involving compromised DA systems. -

Principal Investigator: **KANG, UN Jung**
Grant Number: **5R01NS032080-11**
Title: **Dopamine regulation in parkinsonian rat by gene therapy**

Abstract: L-3,4-dihydroxyphenylalanine (L-DOPA) is the mainstay of therapy for Parkinson's disease (PD). Chronic L-DOPA therapy is limited, however, by the development of motor response complications, such as progressively shorter duration of improvement in akinesia (wearing-off) and the appearance of L-DOPA-induced abnormal involuntary movements. Innovative methods of sustained and localized central nervous system (CNS) dopamine delivery may further optimize L-DOPA therapy. Such methods are being explored clinically by CNS transplantation studies with fetal dopaminergic neurons and experimentally by neuronal stem cell implants and gene therapy. Our studies during the past funding cycles have defined optimal sets of genes necessary for dopamine replacement using ex vivo gene therapy using genetically modified fibroblasts. We also developed rat behavioral models that are relevant to the akinesia of PD patients. Using akinesia behaviors, we have noted that lesion severity has a major influence on the shortening of the response duration with minor contribution by the chronic intermittent L-DOPA therapy. Therefore, studies proposed in this continuing renewal application will determine the optimal parameters of gene therapy to improve akinesia and minimize and prevent motor response complications. We will use adeno-associated virus vectors to deliver tyrosine hydroxylase and guanosine triphosphate (GTP) cyclohydrolase 1 genes. The optimal combination of anatomical targets for gene therapy to improve akinesia will be defined by examining the effects of gene therapy delivered to basal ganglia structures, such as subthalamic nucleus, substantia nigra par reticulata, that receive dopaminergic inputs, in addition to the striatum. The optimal timing to initiate dopamine replacement gene therapy to forestall development of motor response complications will also be examined. These results will have significant implications beyond dopamine replacement gene therapy proposed here and guide other therapies such as fetal dopaminergic cell transplantation, neurotrophic factor therapy, stem cell therapy, and other CNS targeted delivery systems. -

Principal Investigator: **KIM, KWANG S**
Grant Number: **5R21NS044439-02**
Title: **DA-specific gene discovery and promoter engineering**

Abstract: Gene therapy techniques need substantial development to provide therapeutic possibilities for treating neurological disorders such as Parkinson's disease (PD). Based on molecular control mechanisms of noradrenergic neuron-specific gene regulation, we recently devised a gene delivery system that can efficiently target transgene expression to noradrenergic neurons in a cell-specific manner. Our long-term goal is to establish gene therapy system(s) that will drive efficient transgene expression in a dopamine (DA) neuron-specific fashion based on discovery and characterization of DA-specific genes. Toward this end, we propose to identify and isolate genes that are selectively expressed in the DA mid-brain area by analyzing gene expression profiles using the most comprehensive cDNA microarrays such as the augmented NIA 16K chip and augmented RIKEN 16 K chip. Because these chips do not cover the whole genome yet, we will also identify novel DA-specific genes by the PCR-based subtractive hybridization techniques. Expression patterns of putative DA-specific genes will be tested by semi-quantitative RT-PCR using independently isolated mRNAs, and will be confirmed by in situ hybridization. Among the isolated DA-specific genes, we will first focus on putative DNA-binding transcription factors. The consensus binding sites for these putative transcription factors will be defined and their potential promoter function will be tested by cotransfection assays using cell line systems. On the basis of the mechanism of action of the novel DA-specific transcription factor(s), synthetic promoters will be developed and optimized. The optimized synthetic promoter will be subcloned in front of the reporter lacZ gene in the context of the self-inactivated lenti viral vectors. Cell type-specific expression of the reporter gene will be examined using both in vitro mesencephalic primary neuronal cultures as well as in different rat brain areas following stereotactic injection. At the later stage of this proposal, we will plan to use our developed promoter system(s) to deliver therapeutic genes (e.g., GDNF and Bcl 2) to the DA neurons and will test whether they can efficiently ameliorate behavioral symptoms in animal models of PD. The proposed research will identify and isolate genes that are selectively expressed in the mid-brain DA area on a genome-wide scale and will characterize their transcriptional regulation. Based on these mechanisms, we will devise novel and innovative DA-specific promoter systems and test them using in vitro and in vivo systems. In combination with safe viral vectors, our developed gene delivery systems can be translated clinically into gene therapy approaches for PD and other neurological disorders, in which DA

Principal Investigator: KORDOWER, JEFFREY H
Grant Number: 5R01NS043290-03
Title: DYSKINESIAS IN LENTI-GDNF TREATED PARKINSONIAN MONKEYS

Abstract: Fetal nigral grafts can cause "runaway" dyskinesias in patients with Parkinson's disease (PD;Freed et al., 2001). These dyskinesias are severe, debilitating and strongly indicate that 1) novel dopaminergic surgical therapeutic strategy planned for clinical trials need to be tested preclinically for their effects upon dyskinesias and 2) the mechanisms underlying these dyskinesias need to be elucidated. We have recently demonstrated that lentiviral gene delivery of glial cell-derived neurotrophic factor (GDNF) potently prevents motor dysfunction and prevents nigrostriatal degeneration in nonhuman primate models of PD (Kordower et al., 2000). Prior to initiating clinical trials with lenti-GDNF, it effects upon dyskinesias need to be evaluated in parkinsonian monkeys. Freed, Fahn and coworkers (2001) have hypothesized that grafted-mediated dyskinesias result from graft overgrowth. However, their own PET and post-mortem data, as well as the data from others (Kordower et al., 1995, Lee et al 1999), do not support this view. We propose an alternative hypothesis that these dyskinesias result from local "hot spots" of hyperdopaminergic function interacting with the levodopa primed brain. We plan to test this hypothesis by comparing gene therapies that induce either a) widespread or b) local hyperdopaminergic function upon dopa-induced dyskinesias and the role of dopa priming. This application will have three Specific Aims. Specific Aim 1 will test the hypothesis that lenti-GDNF treatment to non-levodopa primed MPTP-treated monkeys will prevent, or diminish the intensity of dyskinesias when they are later treated with levodopa. Specific Aim 2 will test the hypothesis that lenti-GDNF will diminish the dyskinesia profile in dyskinesic MPTP-treated monkeys previously primed with levodopa. Specific Aim 3 will test the hypothesis that "hot- spot" hyperdopaminergic function, but not homogenous hyperdopaminergic innervation, will enhance the dyskinesia profile of parkinsonian monkeys and that elimination of GDNF will reverse the functional and dyskinesic effects established previously by this trophic factor. The study of dyskinesias has become a compelling area of PD research. Exciting therapeutic strategies such as gene therapy need to be evaluated for their effects on dyskinesias so that they are both safe and effective. This application will determine whether potent dopaminergic gene therapies influence dyskinesias in the best animal model of PD.-

Principal Investigator: LANSBURY, PETER T
Grant Number: 1R21NS047420-01A1
Title: High Throughout Assay to Probe UCH-L1 Ligase Inhibitors

Abstract: Parkinson's disease (PD) is characterized by the presence of Lewy bodies (the cytoplasmic neuronal inclusions) and the significant loss of dopaminergic neurons in the substantia nigra, a-synuclein was identified as one major fibril component of the Lewy bodies, thus linked the accumulation of this protein to the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of
the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of
degradation process, can also contribute to the build-up and consequently fibrillation of the protein. A gene, PARK5, has been linked to PD are involved in proteasomal degradation pathway and it is an ubiquitin C terminal hydrolase (UCH-L 1) that hydrolyzes C-terminal ester and amides of ubiquitin and is believed to play a key role in processing polyubiquitin and/or ubiquity lated proteolytic peptide. A rare mutation (193M) of UCH L 1 that yields a 50% reduction in its hydrolytic activity has been tentatively linked to a rare early onset form of PD, at the same time a polymorphism of the enzyme (S 18Y) was indicated to reduce the risk of PD. The assumption that each enzyme expresses a single enzymatic activity in vivo, however, is challenged by the linkage of UCH-L 1 to PD. UCH-L 1, especially those variants linked to higher susceptibility to PD, causes the accumulation of a-synuclein in cultured cells, an effect that cannot be explained by its recognized hydrolase activity. UCH-L1 exhibits a second, dimerization-dependent, ubiquityl ligase activity. The polymorphic variant of UCH-L1 that is associated with decreased PD risk (S 18Y) has reduced ligase activity, but comparable hydrolase activity as the wild-type enzyme. Thus the ligase activity, as welt as the hydrolase activity of UCH-L1 may play a role in proteasomal protein degradation, a critical process for molecules ("molecular probes") that can be used to perturb UCH-L1 ligase activity in cell culture and animal models of PD. This "chemical genetic" strategy is complementary to traditional genetic approaches (e.g., knockouts and trasngenics) for understanding protein function but has a distinct advantage in that the probes are potential lead compounds for the development of novel PD therapetutics. The program detailed below will seek probes with the following activities: (1) inhibitors ofUCH-L1 dimerization, (2) inhibitors of UCH-L1 ligase activity, and (3) repressors and activators of UCH-L1 expression. -

Principal Investigator: LI, SENLIN
Grant Number: 1R01NS046004-01A1
Title: Macrophage Gene Therapy of Neurodegenerative Diseases

Abstract: Neurodegenerative diseases affect a large population of patients. Existing therapies are not satisfactory. Gene therapy holds promise, but focal delivery of DNA and the level of gene expression are challenging. Macrophages are recruited from bone marrow to most tissues of the body including the CNS, thus making them an attractive option for gene delivery. Galactosialidosis (GS) has been corrected by bone marrow-derived macrophages expressing human protective protein/cathepsin A (PPCA) transgene in a mouse model (PPCA-/-). However, correction in the CNS was incomplete due in part to weakness of the CSF-1R promoter used in the study. We have developed a series of super macrophage promoters (SMP) that are up to l00-fold stronger in vitro than the CSF-1R promoter. In models of the highly prevalent Parkinson's disease (PD), local delivery of glial cell line-derived neurotrophic factor (GDNF) has been found beneficial. We hypothesize that highly effective CNS delivery of GDNF can be achieved with the use of our super macrophage promoters and this will greatly ameliorate the pathologic changes and neurological defects in animal models of PD. To explore this hypothesis, our specific aims are: 1) To characterize these super macrophage promoters by transplantation of bone marrow stem cells transduced ex vivo with lentiviral vectors and in transgenic mice using EGFP (enhanced green fluorescent protein) as a reporter. Promoters with the greatest strength and tissue-specificity for macrophages will be used in the subsequent aims. 2) To ameliorate neurodegeneration in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease by syngeneic transplantation of HSC transduced ex vivo with lentivectors expressing GDNF gene in macrophages/macroglia driven by the SMP. Bone marrow stem cells will be transduced ex vivo with GDNF expressing lentivirus and transplanted into lethally irradiated recipient mice. Four weeks after bone marrow transplantation, the recipient mice will be injected subcutaneously with MPTP. At selected time points post MPTP administration, PET scan and behavioral testing will be performed, and brain tissue will be examined for dopamine uptake and expression of tyrosine hydroxylase (TH). In the substantia nigra pars compacta (SN), dopaminergic neurons will be counted and cell apoptosis will be assessed by TUNEL staining and immunohistochemistry for active easpase-3. 3) To ameliorate neurodegeneration in the same way as in Aim 2, but GDNF expression will be controlled by a tetracycline-regulatable gene expression system. To evaluate the effects of macrophage/ super promoter-mediated delivery and expression of GDNF on degenerating

Principal Investigator: LOWENSTEIN, PEDRO R
Grant Number: 5R01NS042893-03
Title: Gene Therapy for Neurological Diseases

Abstract: Parkinson's disease (PD) is a chronic neurodegenerative disorder. Although we do not yet understand its cause, there is extensive degeneration of nigro-striatal DA neurons. Powerful neurotrophic factors, which could be used for the treatment of Parkinson's disease, have been described recently. The ultimate goal of this proposal is to develop novel high-capacity adenoviral systems for long term, stable, non-cytotoxic, and non-immunogenic delivery of neuroprotective genes to the brain for both experimental transgene expression in adult animals, and for the future treatment of chronic neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease by gene therapy. Currently used adenoviruses are efficient vectors for gene transfer into the brain. However, their use has been limited by high doses of vectors needed, vector-cytotoxicity, short-term transgene expression, and deleterious effects of anti-adenovirus inflammatory and immune responses. These limitations are due to: (i) low efficiency of transcriptional promoter elements currently used, which directly leads to the need to use higher doses of vectors, and (ii) cytotoxicity and immunogenicity of viral proteins expressed from the genomes of first generation adenoviral vectors. We now wish to initiate the next stage in the design of efficient, safe, long-term expressing vectors for neurological gene therapy. First, high-capacity helper-dependent adenoviral vectors that express no adenoviral genes will be constructed. Second, the amount of protein produced per input virus will be increased by using stronger promoters alone, or combined with enhancer elements. Third, the efficacy of the new vectors will be tested, by expressing selected neuroprotective genes and verify that they are effective in a model of neurodegeneration. The reagents and principles established by this work will be of substantial value to those with interests in the basic and clinical neurosciences, and will lead to the development of novel approaches to the treatment of chronic neurodegenerative diseases. This research will facilitate the development of the tools needed to achieve long-lived, safe, non-cytotoxic transgene expression, and, ultimately, for the treatment of patients suffering from chronic neurodegenerative disorders. -

Principal Investigator: Mouradian, Maral
Grant Number: 5Z01NS002826-14
Title: Molecular Pathogenesis Of Cell Death In Neurodegenerative Diseases

Abstract: Unavailable

Principal Investigator: MUZYCZKA, NICHOLAS
Grant Number: 2P01NS036302-06A1
Title: Adeno-Associated Virus Gene Transfer to Nervous System

Abstract: The long term goal of this Program is to develop gene transfer methods for the treatment of neural disorders. Three groups that are well integrated have come together to develop methods for using recombinant Adeno-associated virus (rAAV) for the treatment of retinal and CNS neurodegenerative diseases. Project 1 (Muzyczka) proposes genetic experiments to identify the proteins in the substantia nigra and striatum that interact with alpha synuclein. It will specifically examine alpha syn interactions with GRK and PLD2, and develop for the first time somatic knockouts of GRK and PLD2 using AAV vectors. It will also examine the effect of oxidative stress in combination with alpha syn overexpression on neurodegeneration in the substantia nigra. Finally, it will use biochemical techniques to directly identify protein complexes that contain alpha syn. Project 2 (Hauswirth and Lewin) will take the next step toward developing a therapy for P23H rhodopsin RP using the ribozymes they developed in the previous grant period. Further, they will test two new strategies for RP that are likely to be of more general use for all RP diseases. The first is the use of GDNF expression to promote photoreceptor survival. The second is to replace all (wild type and mutant) endogenous rhodopsin mRNAs with a wild type mRNA. If successful, this should prove to be a general approach that could be applied to all genetic RP, regardless of the point mutant involved. Project 4 (Mandel) will extend their preclinical experiments toward developing AAV mediated gene transfer for Parkinson disease. Specifically, they will develop regulatable GDNF constructs that are a prerequisite for clinical applications, do the first comprehensive analysis of the immune response to AAV vectors that are injected into the brain, and test their therapeutic GNDF strategy in a primate model of Parkinson's to obtain dosing information and confirmation of efficacy in a brain model closer to human. Two cores are also proposed. Core A (Administration) will continue in its role of providing fiscal/administrative support, educational programs, and program oversight in the form of internal and external advisors. The Vector Core will continue to improve the efficiency and scaleability of rAAV vectors. In addition to providing the routine service of production and purification of rAAV2-based vectors, the Core will also develop methods for purification of alternative AAV serotypes and capsid mutants to be used in projects 1, 2, and 3.-

Principal Investigator: MUZYCZKA, NICHOLAS
Grant Number: 3P01NS036302-06A1S1
Title: Adeno-Associated Virus Gene Transfer to Nervous System

Abstract: Unavailable

Principal Investigator: Oldfield, Edward
Grant Number: 5Z01NS002813-15
Title: Drug Delivery Techniques

Abstract: Unavailable

Principal Investigator: REDMOND, D EUGENE
Grant Number: 1U01NS046028-01A1
Title: GDNF Delivery to MPTP Monkeys by EIAV lentivirus and AAV

Abstract: An effective gene therapy for Parkinson's disease is the goal of this proposal, which will test the effectiveness and safety of human glial cell line derived neurotrophic factor (GDNF) delivered by two improved vector systems derived from equine infectious anemia virus (EIAV) or from adenoassociated virus (AAV). Both vectors deliver the cellular marker gene, nuclear localized lacZ (lacZnl) or GDNF efficiently and stably into nigrostriatal target regions, can be regulated using a tetracycline promoter system, and offer additional safety that the respective wild-type viruses do not cause any disease in humans. The recombinant vectors will be tested in the parkinsonian model produced by the neurotoxin MPTP in monkeys. GDNF has shown promise for preventing or reversing morphological, biochemical and functional deficits in other models of Parkinson's disease in rodents and primates, using rAAV, and rHIV. But these studies also showed important problems to be solved to ensure that a GDNF gene therapy will be safe and effective in patients. Concerns about inflammatory, cytotoxic, inadequate or excessive gene expression, persistence, viral recombination or replication have led to the development of improved and safer vectors with regulatable promoters, which will be tested in this proposed project. Initial studies will address transgene expression (lacZnl or GDNF) in normal African green monkeys, determining effective titers, transduction efficiency, cellular tropism, distribution, level, and stability of transgene expression, neuropathology and host cellular responses after delivery by rEIAV or rAAV. Each of the two vectors will then be used to deliver GDNF to the nigrostriatal system of MPTP parkinsonian monkeys to test hypotheses that GDNF expression will improve function in both moderate and severely parkinsonian monkeys for periods up to 24 months. The most effective procedures will be optimized by comparing injection sites, a regulatable promoter to inactivate gene expression, and safety of all procedures including high injection titers. Measures of efficacy will include behavioral parameters, molecular assays of transgene expression using ELISA for protein, RT-PCR for mRNA and PCR for vector DNA, biochemical assays of DA and its metabolites, neuroanatomical and morphometric analyses, neuropathology, clinical chemistry, SPECT imaging, and autoradiography. These studies aim to provide the necessary data to initiate successful clinical trials in Parkinson's patients at the earliest possible time. -

Principal Investigator: SILVERMAN, RICHARD B
Grant Number: 1R01NS047331-01A1
Title: Celestrols for Treatment of Neurodegenerative Diseases

Abstract: The expression of molecular chaperones has been shown to suppress protein misfolding/aggregation and cellular toxicity phenotypes in model systems associated with Huntington's Disease, Alzheimer's Disease, Parkinson's Disease, and ALS. A feature common to diseases of protein conformation is the appearance of folded intermediates that self-associate to form protein aggregates and inclusions. The molecular chaperones Hsp90 and Hsp70 sequester damaged proteins that appear in cells exposed to physiological and environmental stress. The ability of molecular chaperones to suppress the cellular toxicities associated with expression of these "toxic" proteins may be due to the intrinsic properties of chaperones to capture and suppress the appearance of folded intermediates. Therefore, we propose that the identification of small molecules that elevate the expression of genes encoding heat shock proteins and molecular chaperones should lead to the development of novel therapies beneficial to the prevention of neurodegenerative diseases. The rationale for this proposal is based on results obtained by our laboratory and others who participated recently in a screening program organized by the NINDS, Huntington Disease Society of America, Hereditary Disease Foundation, and the ALSA to identify new drugs for treating these diseases. A search was carried out for drugs that activate the heat shock response; the most effective compound identified was the natural product celastrol. Synthetic analogs of celastrol will be prepared to optimize its effectiveness as a regulator of the heat shock response and a suppressor of neurotoxicity and to determine its mechanism of action as an activator of the heat shock response. To probe the function of celastrol as a potential therapy for neurodegenerative diseases, the following Specific Aims will be addressed: (1) Synthesize analogs of celastrol that induce the human heat shock response using a heat shock promoter-reporter assay in human tissue culture cells. (2) Determine the mechanism of action of celastrol (or an analog). The working model is that celastrol activates the heat shock response by inducing heat shock transcription factor HSF1. The mechanism by which HSF1 activity is induced by celastrol will be determined. It also will be determined whether celastrol, by virtue of its ability to activate the expression of chaperones, can reduce the aggregation and neurotoxicity of the Huntington Q64 protein expressed in a human SH-SY5Y neuroblastoma cell line. (3) Studies will be carried out to identify the binding target for celastrol using molecular biological and biochemical techniques. Identified target(s) will then be cloned and characterized. Results of these studies

Principal Investigator: STAROPOLI, JOHN F
Grant Number: 1F31NS048668-01
Title: Parkin and Its Regulation of Neuronal Apoptosis

Abstract: Mutations in parkin underlie an autosomal recessive form of Parkinson's disease, the second most common neurodegenerative disease. To test a working model of parkin as a component of a multi-subunit, SCF-like ubiquitin ligase complex that protects dopamine neurons from apoptosis, other components of the complex, including sel-10 and cullin-1, will be dowregulated by RNA interference in murine primary neuronal cultures. Downregulation of these components will be evaluated for potentiation of dopamine neuron apoptosis and compared to the effects of downregulating parkin itself. To test the hypothesis that a candidate substrate of the parkin-associated complex, cyclin E, is a key mediator of the apoptotic cascade(s) against which wildtype parkin normally protects neurons, pharmacological inhibition of cyclin E-associated activity will be evaluated for rescue of dopamine neurons in the context of parkin, sel-10, or cullin-1 downregulation. Finally, lentivirus-mediated overexpression of parkin in the same primary culture system will be assessed for protection of dopamine neurons from neurotoxins as compared to overexpression of mutant forms of parkin, including clinically defined mutations and forms deleted in the ubiquitin homology and RING domains.-

Principal Investigator: TKATCH, TATIANA
Grant Number: 1R21NS048524-01
Title: RNAi Targeting of Kv3 Channels in Basal Ganglia Disease

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by impairment of motor function. It affects about 1 in 1000 adults, rising exponentially after the age of fifty. At present there is no treatment for PD shown to definitively attenuate disease progression. Even temporal correction of symptoms extending the period of physical mobility is considered valuable. We suggest to test a new strategy to relieve motor symptoms of the Parkinson's disease. The abnormal correlated rhythmic activity in the globus pallidus (GP) and subthalamic nucleus (STN) are believed to underlie bradykinesia and tremor of PD patients. A specific set of membrane conductances in GP and STN neurons enable such activity. Recent work by our group has shown that high frequency burst discharge in GP and STN neurons is dependent upon their expression of a combination of voltage-dependent Kv3 K+ channel subunits. These neurons form heteromeric channels containing Kv3.1 and Kv3.4 subunits. These heteromeric channels are very efficient at repolarizing spikes - keeping them very brief - and then deactivating after the spike to allow the next spike to occur quickly. Eliminating the Kv3.4 subunit from these channels diminishes the repolarizing efficiency of the channels, resulting in lower maximal discharge rates. Thus our goal is to test the hypothesis that the suppression of Kv3.4 subunit in GP/STN neurons will dramatically reduce pathological, high frequency burst discharge leading to symptomatic relief in PD models and patients. Kv3.4 is an excellent target for gene therapy approaches since its expression is highly specific for fast spiking neurons and the firing of non-targeted neurons in GP/STN surrounding areas should not be affected. We propose to use lentivirus vector to deliver small interfering RNA (siRNA) designed to trigger the degradation of Kv3.4 mRNA in GP and STN neurons. The proposed specific aims will allow development of the technology that is necessary for testing of our hypothesis in the animal models of PD. -

Principal Investigator: WETZEL, RONALD B
Grant Number: 5R01NS046356-02
Title: Conformational antibodies recognizing amyloid epitopes

Abstract: A number of neurological and other diseases are associated with the formation of protein aggregates called amyloid fibrils. Although the protein component of amyloid is different for different diseases, the three-dimensional shape of the amyloid seems to be the same in all cases. It has proven exceedingly difficult to get detailed structural information on amyloid, compromising our efforts to understand and devise treatments for Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, and an array of other devastating conditions. We have developed antibody molecules that have the unique ability to bind to amyloid fibrils, regardless of the amino acid sequence of the constituent protein. Here we propose to develop these conformational antibodies as tools to improve our understanding of the amyloid structure and how it develops in human diseases. The specific aims are to (1) develop additional antibodies and antibody fragments, (2) structurally characterize these antibodies as a novel window onto the structure of amyloid fibrils, (3) characterize the fundamental basis of the antibody-amyloid interaction, and (4) develop the antibodies into tests for detecting amyloid in tissue and serum, as well as to monitor the emergence of the amyloid motif during in vitro amyloidogenesis. The tools involved in this work will include hybridoma and phage display technology, recombinant expression, mutagenesis, protein modeling and crystallography, immunochemical binding assays, and in vitro amyloid fibril assembly reactions. Since recently described vaccine approaches to amyloid diseases such as AD depend on the generation of antibodies, characterizing the structural basis of the anti-amyloid reaction is important. It is also important to understand the structure of amyloid and how these fibrils grow, as a means toward developing agents that will compromise fibril cytotoxicity and fibril growth. Finally, as a ubiquitous alternative folding pattern of protein polymers, amyloid and its structure is of fundamental importance to our understanding of the molecular basis of life. -

Principal Investigator: XU, ZUOSHANG
Grant Number: 1R01NS048145-01
Title: Understanding mechanism and therapy of ALS using RNAi

Abstract: Diseases caused by dominant, gain-of-function mutations develop in people bearing one mutant and one wild-type copy of the gene. Some of the best known examples of this class are neurodegenerative diseases, including Huntington's, a subset of amyotrophic lateral sclerosis (ALS), Alzheimer's and Parkinson's diseases. In all these diseases, the exact pathways whereby the mutant proteins cause cell degeneration are not entirely clear, but the origin of the cellular toxicity is known to be the mutant protein. Thus, selectively lowering or eliminating the mutant protein is a key step in developing effective therapies. Until recently, it was not clear how specific down-regulation of a wide variety of mutant proteins could be achieved. But now, new advances in RNA interference (RNAi) raise the possibility that RNAi can be developed and eventually applied as a therapeutic means for these neurodegenerative diseases. RNAi can mediate sequence-selective suppression of gene expression in a wide variety of eukaryotes by introducing short RNA duplexes (called small interfering RNAs or siRNAs) with sequence homologies to the target gene. Recent experiments indicate that small hairpin RNAs (shRNAs) transcribed in vivo can trigger degradation of corresponding mRNAs similar to siRNA. These developments raise the possibility that siRNA duplexes or vectors expressing shRNAs may be used to block the expression of a toxic mutant gene. This proposal investigates in vivo efficacy of RNAi therapy using transgenic technology in a mouse model for ALS that is caused by mutations in Cu, Zn superoxide dismutase (SOD1). To determine the potential of RNAi therapy, we will express shRNAs targeting specifically the mutant mRNAs in transgenic mice. We will test how effective and how specific these shRNAs are in suppressing the mutant protein expression and alleviating the disease. To determine in which cell types the suppression of the mutant expression is most crucial, we will express shRNAs in selected cell types using Cre-lox recombination system, We will determine in which cell type suppression of mutant SOD1 expression has the largest impact in alleviating disease. To determine the optimal time for therapy, we will use the Tamoxifeninducible Cre recombinant system to determine at what stage of the disease induction of shRNA to suppress mutant SOD1expresion is most effective.-

Principal Investigator: **YUREK, DAVID M**
Grant Number: **5R01NS042862-03**
Title: **Gene Therapy, Neural Grafts & Parkinson's Disease**

Abstract: Clinical trials have provided encouraging evidence that grafts of fetal dopamine neurons are an effective therapeutic approach toward counteracting the symptoms of Parkinson's disease. Modest therapeutic benefits are observed in grafted patients despite clinical and experimental evidence that survival of grafted cells is low and graft reinnervation is incomplete. The poor survival and limited fiber outgrowth may be a consequence of neural grafts placed ectopically into an environment where the grafted neurons do not receive the proper signals for successful growth and integration into the neural circuitry of the host brain. Gene therapy may be a viable technique to introduce factors [neurotrophic factors] into brain tissue that can potentiate the survival and functional outgrowth of neural grafts, and thus improve the therapeutic value of the graft. In the proposed studies, regulated viral vectors will be injected into the lesioned nigrostriatal pathway of rodents with experimental Parkinson's disease in order to induce transgene expression of several neurotrophic factors that have a history of providing potent neurotrophic support for dopamine neurons. Subsequently, neural grafts will be implanted into lesioned/transduced brain sites and the survival, reinnervation, and function of the grafts will be assessed. Because Parkinson's disease has a higher incidence in the elderly than in the younger population, and recent experimental evidence suggests that the expression of endogenous neurotrophic factors are diminished in the aged striatum following a neurodegenerative lesion, experiments will be performed in young, middle-age, or old rats with experimental Parkinson's disease and the results will be compared within and between each age group. The studies are designed to determine the optimal temporal expression of neurotrophic factors [GDNF, BDNF, FGF-2] that improve graft development and function using regulated viral neurotrophic factors [GDNF, BDNF, FGF-2] that improve graft development and function using regulated viral vectors in young and aged animals with experimental Parkinsonism. These studies will also determine if combinations of viral vectors expressing different neurotrophic factors can be used to improve the therapeutic effects of dopamine grafts.-